Elucidating the putative link between prefrontal neurotransmission, functional connectivity, and affective symptoms in irritable bowel syndrome

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Supplementary information

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Supplementary table S1

Sample characterization and group comparisons of clinical characteristics and psychological measures.

	IBS (N = 64)	HCs (N = 32)	*p*
Age (years)	30.00 (24.25 – 36.75)	32.5 (24.00 – 45.25)	0.330
Intestinal symptoms (IBS-SSS)	329.00 (275.00 – 384.00)	4.00 (0.00 – 23.00)	< 0.001
Pain intensity (BPI)	15.00 (11.00 – 21.50)	0.00 (0.00 – 0.00)	< 0.001
Pain interference (BPI)	32.00 (11.00 – 45.50)	0.00 (0.00 – 0.00)	< 0.001
Symptom-specific anxiety (VSI)	44.50 (32.00 – 57.00)	1.00 (0.00 – 3.00)	< 0.001
Anxiety (HADS)	10.00 (8.00 – 14.00)	3.50 (2.00 – 5.00)	< 0.001
Depression (HADS)	5.00 (3.00 – 8.75)	1.00 (0.00 – 2.00)	< 0.001

Data are given as median and interquartile ranges are provided. Abbreviations: BPI, brief pain inventory; HADS, Hospital anxiety and depression scale; HCs, Healthy Controls; IBS, Irritable Bowel Syndrome; IBS-SSS, IBS symptom severity score intestinal; VSI, visceral sensitivity index.

Supplementary information S2

a) Group comparisons of neurotransmitter levels excluding patients on antidepressant treatment

To exclude possible confounding effects of antidepressant medication on mPFC neurotransmission, additional analyses were performed in which 21 patients who had reported SSRI or low-dose TCA medication intake at the time of participation were excluded. Mann-Whitney *U*-tests comparing only patients free from antidepressant medication (N = 43) with HCs (N = 32) confirmed no group differences in either GABA+ or Glx concentrations (Supplementary table S2).

Table S2: Comparison of mPFC GABA+ and Glx concentrations in patients free from antidepressant treatment and HCs.

	IBS (N = 43)	**HCs (N = 32)**	***p***
mPFC GABA+	0.71 (0.66 – 0.76)	0.71 (0.62 – 0.75)	0.509
mPFC Glx	4.71 (4.01 – 5.12)	4.44 (3.81 – 5.23)	0.495

Data are given as median and interquartile ranges are provided. Abbreviations: mPFC, medial prefrontal cortex; GABA+, γ-aminobutyric acid+ macromolecule signal; Glx, glutamate+glutamine.

b) Subgroup comparisons of GABA+ levels excluding patients on antidepressant treatment

As performed in the full sample, women with IBS and free of antidepressant treatment were sub-grouped based on HADS anxiety scores, resulting in groups of 18 IBS^{+} patients (*i.e.*, 11 patients in this group were excluded due to medication intake) and 25 IBS^{-} patients (*i.e.*, 9 patients were excluded). These data were subjected to a Kruskal-Wallis test followed by post hoc *U*-tests. Kruskal-Wallis-test revealed a trend-level group effect ($X^2 = 4.802$; $p < 0.10$).

Exploratory post hoc *U*-tests supported enhanced GABA+ levels in IBS+ compared to IBS- patients ($U = 140.00$; $p = 0.039$) and a trend towards higher GABA+ levels in IBS with high anxiety symptom severity compared to HCs ($U = 200.00$; $p = 0.077$), essentially confirming the results in the full sample while failing to reach statistical significance after correction for multiple comparisons.

Supplementary table S3

Group comparisons of mPFC N-acetylaspartate, Creatine and Choline concentrations.

	IBS (N = 64)	HCs (N = 32)	*p*
N-acetylaspartate	4.95 (4.53 – 5.89)	4.75 (4.18 – 5.44)	0.098
Creatine	5.00 (4.41 – 5.58)	4.51 (4.00 – 5.46)	0.105
Choline	1.04 (0.94 – 1.19)	0.99 (0.87 – 1.23)	0.408

Data are given as median and interquartile ranges are provided. Abbreviations: HCs, Healthy Controls; IBS, Irritable Bowel Syndrome.

Supplementary table S4

Correlations between neurotransmitter concentrations, clinical characteristics, and psychological measures.

	Age	GABA+	Glx	Anxiety	Depression	GI-specific anxiety	Symptom severity	Pain intensity	Pain interference
Age	1	-0.089	-0.044	-0.144	-0.009	-0.184	-0.150	-0.018	-0.092
	96	0.425	0.698	0.198	0.937	0.099	0.180	0.874	0.412
GABA+		1	0.094	**0.280****	**0.222***	0.149	0.144	0.083	0.066
		96	0.399	**< 0.01**	**< 0.05**	0.181	0.198	0.460	0.559
Glx			1	0.070	-0.038	0.066	0.152	0.097	0.098
			96	0.534	0.732	0.553	0.174	0.386	0.379
Anxiety				1	**0.805*****	**0.735*****	**0.715*****	**0.724*****	**0.735*****
				96	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Depression					1	**0.659*****	**0.650*****	**0.686*****	**0.721*****
					96	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
GI-specific anxiety						1	**0.838*****	**0.769*****	**0.752*****
						96	**< 0.001**	**< 0.001**	**< 0.001**
Symptom severity							1	**0.809*****	**0.839*****
							96	**< 0.001**	**< 0.001**
Pain intensity								1	**0.881*****
								96	**< 0.001**
Pain interference									1
									96

Spearman’s rank correlations between Glx and GABA+ concentrations, psychological and disease-related measures conducted in the full sample. Abbreviations: GABA+, γ-aminobutyric acid+ macromolecule signal; GI, gastrointestinal; Glx, glutamate+glutamine.